PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12133PC2-MLE/AKB	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. **PCT/AU2004/001006**	International filing date *(day/month/year)* 28 July 2004	Priority date *(day/month/year)* 28 July 2003
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] C12N 5/06, C12N 5/08		
Applicant QUEENSLAND UNIVERSITY OF TECHNOLOGY et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      - [ ] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:

[X]	Box No. I	Basis of the report
[ ]	Box No. II	Priority
[ ]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[ ]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
[X]	Box No. VI	Certain documents cited
[ ]	Box No. VII	Certain defects in the international application
[X]	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
7 February 2005	2 November 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **GARETH COOK** Telephone No. (02) 6283 2541

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International application No.
PCT/AU2004/001006

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on translations from the original language into the following language which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1 (b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

☐ the international application as originally filed/furnished

☒ the description:

pages **1-35** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

☒ the claims:

pages as originally filed/furnished
pages* as amended (together with any statement) under Article 19
pages* **36-38** received by this Authority on **7 February 2005** with the letter of **7 February 2005**
pages* received by this Authority on with the letter of

☐ the drawings:

pages **1-6** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages
☐ the claims, Nos.
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to the sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages
☐ the claims, Nos.
☐ the drawings, sheets/figs
☐ the sequence listing (*specify*):
☐ any table(s) related to the sequence listing (*specify*):

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-36	YES
	Claims		NO
Inventive step (IS)	Claims	1-36	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-36	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purposes of this report:

D1 US 5,292,655 (Wille, Jr.) 8 March 1994
D2 US 5,834,312 (Wille, Jr) 10 November 1998
D3 WO 2000/027996 A (Consorzio per la gestione del centro di biotechnologie avanzate et al.) 18 May 2000
D4 Onishi, T., et al., 1999, *Archives of Oral Biology*, 44(4):361-371
D5 Chapinyo, K. et al., 2002, *Journal of Orthopaedic Research*, 20:1070-1078
D6 Nielsen, F. C. and Gammeltoft, S., 1988, *Biochemical and Biophysical Research Communications*, 154(3):1018-1023

The present invention relates to a method of culturing keratinocytes using a serum-free cell culture medium that comprises either insulin like growth factor (IGF)-I or IGF-II. In a preferred embodiment, keratinocytes are cultured in the presence of protein complexes comprising IGF-II and vitronectin (VN) or IGF-I, IGBP and VN.

Novelty (N) and Inventive Step (IS)

D1-D6 describe various culture mediums for the culture of epithelial cells, chondrocytes, mesenchymal stem cells, dental pulp cells and pheochromocytoma cells. The culture media described comprise IGF-I or IGF-II in the absence of serum. However, the culture media described in **D1-D6** do not comprise vitronectin. As such, the subject matter of the present claims is new and meets the requirements of Article 33(2) PCT with regard to novelty.

Claims 1-36 also meet the criteria set out in PCT Article 33(3) with regard to the requirement of Inventive Step because the prior art does not obviously suggest to a person skilled in the art cell culture medium comprising:

(i) at least one IGF selected from IGF-I and IGF-II
(ii) vitronectin or a fragment thereof; and
(iii) an absence of serum or an amount of serum which in the absence of said at least an IGF would not support cell growth.

Industrial Applicability (IA)

The invention defined in the claims is considered to meet the requirements of Industrial Applicability under Article 33(4) of the PCT because it can be made by, or used in, industry.

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Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*
WO 2003/102134	11/12/2003	22/05/2003	28/05/2002

With regard to the document(s) listed in Box VI under "certain documents cited", these are documents published prior to the international filing date but later than the priority date claimed but which would otherwise be considered to be of particular relevance.

Under the PCT, novelty is considered only in respect of documents published before the priority date. The relevance of a document published after the priority date is dependent upon national law. Such documents are excluded from consideration in preliminary examination, under the PCT Guidelines but have been included here for information.

WO 2003/102134 describes a method for expanding mammalian acinar cells comprising culturing the cells in a cell culture system comprising a cell culture medium and cell attachment surface. The cell culture medium disclosed includes soluble active factors including EGF, bFGF, IGF1 and IGF2 (see paragraph [00024], Table 1, Table 2), and the cell attachment surface comprises a surface comprising at least one ECM eg collagen IV, vitronectin or fibronectin (see paragraph [00010]).

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The claims are not fully supported by the description because the claims are not limited to the technical features of the invention described in the specification,.

It appears that the present invention is based on the discovery that culture media comprising IGF-II and VN or IGF-I, IGFBP and VN stimulate significant proliferative responses in primary cultures of keratinocytes in the absence of serum (p. 5 lines 20-23). In particular, the present specification describes the culture of primary keratinocytes in the presence of isolated growth factor complexes that comprise IGF-II and VN or IGF-I, IGFBP and VN (Example 1, 2), and indicates that keratinocytes grown under these conditions were *found to expand more rapidly than those grown using only current best clinical practice* (p. 29 lines 8-10).

Thus it is considered that the present specification provides support for the use of cell culture media comprising isolated growth factor complexes of

(A)IGF-II and VN; or
(B) IGF-I, IGFBP and VN

for the culture of keratinocytes.

The present claims are not limited to the use of a cell culture media comprising the isolated growth factor complexes listed in (A) and (B) above for the culture of keratinocytes. As such, the claims are not fully supported by the description.

CLAIMS

1. A mammalian cell culture medium comprising:

(i) at least one IGF selected from IGF-I and IGF-II;

(ii) vitronectin (VN) or a fragment thereof; and

(iii) an absence of serum or an amount of serum which in the absence of said at least an IGF would not support cell growth.

2. The mammalian cell culture medium of Claim 1, wherein serum is absent or present to a concentration no more than 1% (v/v).

3. The mammalian cell culture medium of Claim 2, wherein serum is present to a concentration no more than 0.5% (v/v).

4. The mammalian cell culture medium of Claim 3, wherein serum is present to a concentration no more than 0.1% (v/v).

5. The mammalian cell culture medium of Claim 1, wherein serum is absent.

6. The mammalian cell culture medium of Claim 1, wherein the IGF is IGF-II.

7. The mammalian cell culture medium of Claim 1, wherein the IGF is IGF-I.

8. The mammalian cell culture medium of Claim 7, further comprising an IGFBP selected from the group consisting of IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5 and IGFBP6.

9. The mammalian cell culture medium of Claim 8, wherein the IGFBP is selected from the group consisting of IGFBP3 and IGFBP5.

10. The mammalian cell culture medium of Claim 9, wherein the IGFBP is IGFBP5.

11. The mammalian cell culture system of Claim 1, wherein the VN fragment does not comprise a heparin binding domain (HBD).

12. The mammalian cell culture system of Claim 11, wherein the VN fragment comprises a polyanionic region.

13. The mammalian cell culture system of Claim 12, wherein the VN fragment is capable of binding an α_v integrin receptor.

14. The mammalian cell culture system of Claim 13, wherein the VN fragment is capable of binding an integrin receptor selected from an $\alpha_v\beta_3$ integrin or an $\alpha_v\beta_5$ integrin.

15. The mammalian cell culture system of Claim 1, wherein vitronectin (VN) is purified autologous vitronectin (VN).

16. The mammalian cell culture medium of Claim 1 comprising IGF-I, an IGFBP and vitronectin in the form of an isolated protein complex.

17. The mammalian cell culture medium of Claim 1 comprising IGF-II and vitronectin in the form of an isolated protein complex.

18. The mammalian cell culture medium of Claim 15 or Claim 16, wherein the isolated protein complex is a synthetic chimeric protein.

19. The mammalian cell culture medium of Claim 1, further comprising one or more other biologically active proteins that promote cell growth and/or differentiation.

20. The mammalian cell culture medium of Claim 19, wherein said another growth factor is EGF and/or bFGF.

21. The mammalian cell culture medium of Claim 1, when used to culture epithelial cells.

22. A mammalian cell culture system comprising a culture vessel and the mammalian cell culture medium of any one of Claims 1-20.

23. The mammalian cell culture system of Claim 22, comprising vitronectin and/or fibronectin, or a fragment thereof, immobilized, bound or otherwise associated with the culture vessel.

24. A method of cell culture including the step of culturing one or more cells in the mammalian cell culture system of Claim 22 or Claim 23.

25. The method of Claim 24, wherein feeder cells are absent for at least part of the duration of culture.

26. The method of Claim 24, wherein the one or more cells are epithelial cells.

27. The method of Claim 26, wherein the one or more cells are keratinocytes or keratinocyte progenitors.

28. The method of Claim 26, wherein the one or more cells are corneal cells.

29. A pharmaceutical composition for aerosol delivery of keratinocytes or keratinocyte progenitor cells comprising one or more keratinocytes cultured according to the method of any one of Claims 24-28, together with a pharmaceutically acceptable carrier, diluent or excipient.

30. The pharmaceutical composition of Claim 29, further comprising a propellant.

31. The pharmaceutical composition of Claim 30, further comprising a fibrin glue.

32. The pharmaceutical composition of Claim 31, further comprising at least an IGF selected from IGF-I and IGF-II.

33. The pharmaceutical composition of Claim 32, comprising IGF-I, an IGFBP and vitronectin or a fragment thereof in the form of an isolated protein complex.

34. The pharmaceutical composition of Claim 32, comprising IGF-II and vitronectin or a fragment thereof in the form of an isolated protein complex.

35. A method of delivering keratinocytes or keratinocyte progenitor cells for skin regeneration *in situ* including the step of spraying the pharmaceutical composition of any one of Claims 29-33 onto the skin of an individual to facilitate skin regeneration.

36. The method of Claim 35, further including the step of growing said keratinocytes or keratinocyte progenitor cells to form regenerated skin *in situ*.